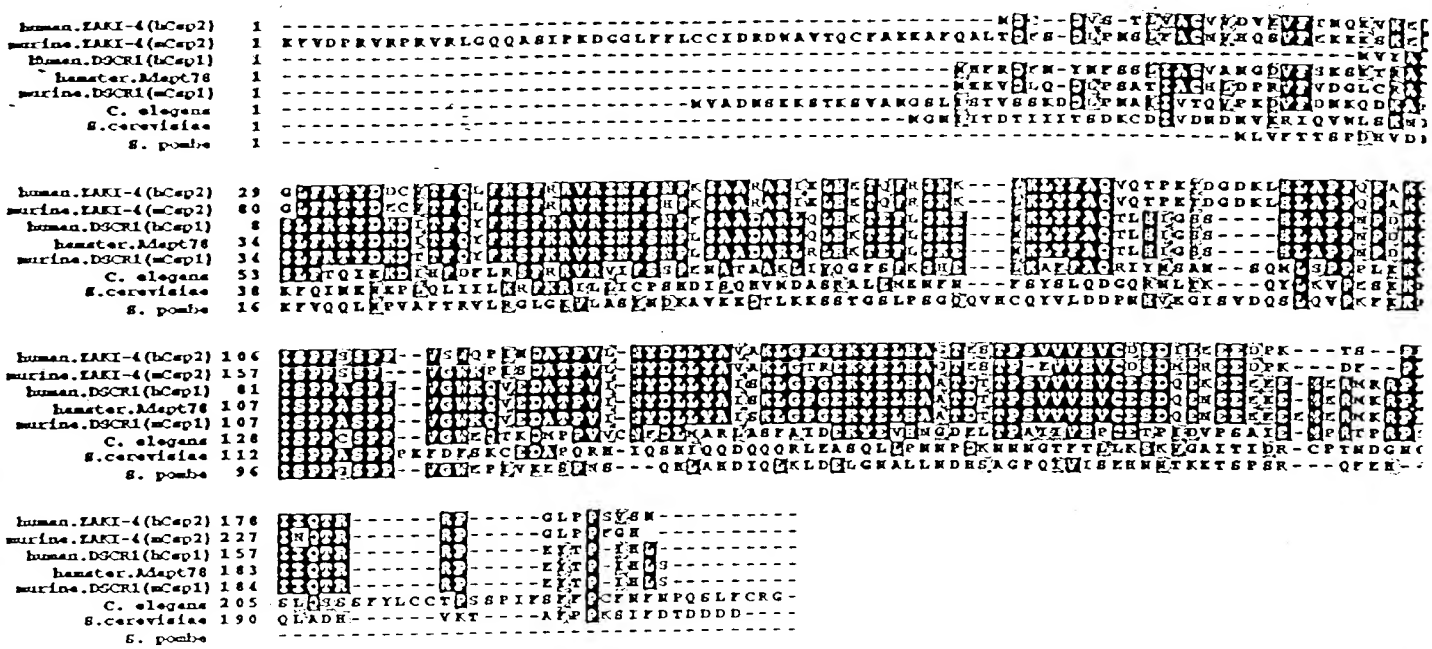
A.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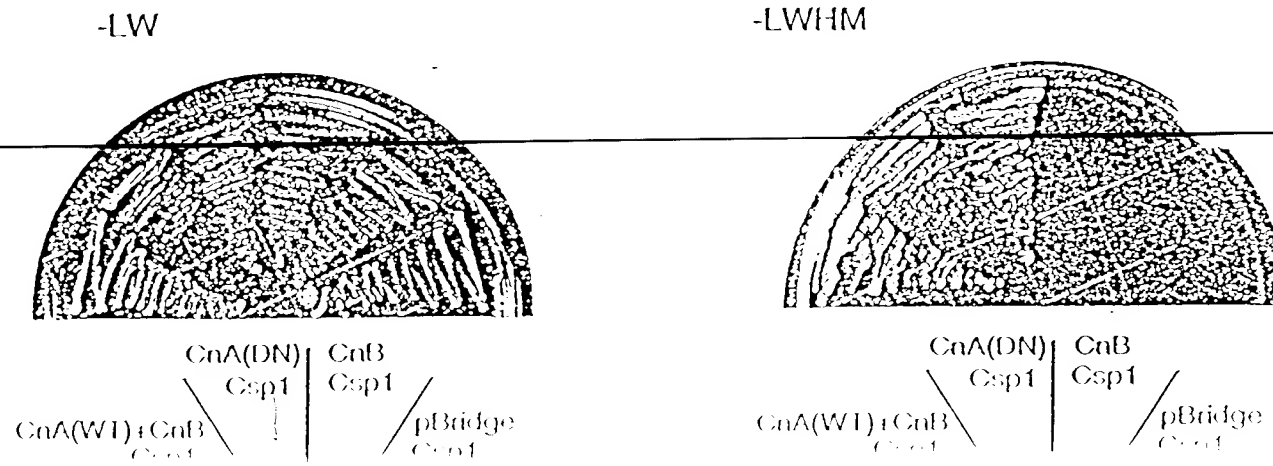
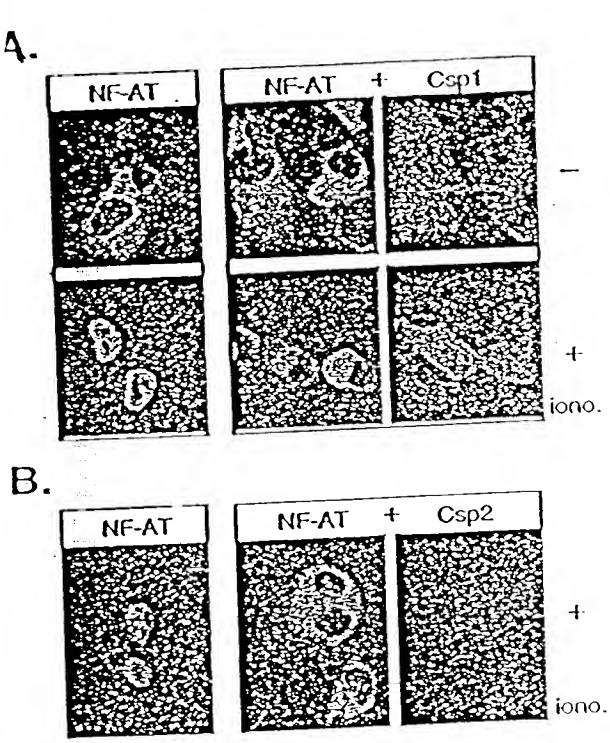
murine.ZAKI-4(mCsp2) 1
human.DSCR1(hCsp1) 1
hamster.Adapt78 1
murine.DSCR1(mCsp1) 1
C. elegans 1
S.cerevisiae 1
S. pombe 1
human.ZAKI-4(hCsp2) 29
murine.ZAKI-4(mCsp2) 80
human.DSCR1(hCsp1) 8
hamster.Adapt78 34
murine.DSCR1(mCsp1) 34
C. elegans 53
S.cerevisiae 38
S. pombe 16
human.ZAKI-4(hCsp2) 106
murine.ZAKI-4(mCsp2) 157
human.DSCR1(hCsp1) 81
hamster.Adapt78 107
murine.DSCR1(mCsp1) 107
C. elegans 128
S.cerevisiae 112
S. pombe 96
human.ZAKI-4(hCsp2) 176
murine.ZAKI-4(mCsp2) 227
human.DSCR1(hCsp1) 157
hamster.Adapt78 183
murine.DSCR1(mCsp1) 184
C. elegans 205
S.cerevisiae 190
S. pombe

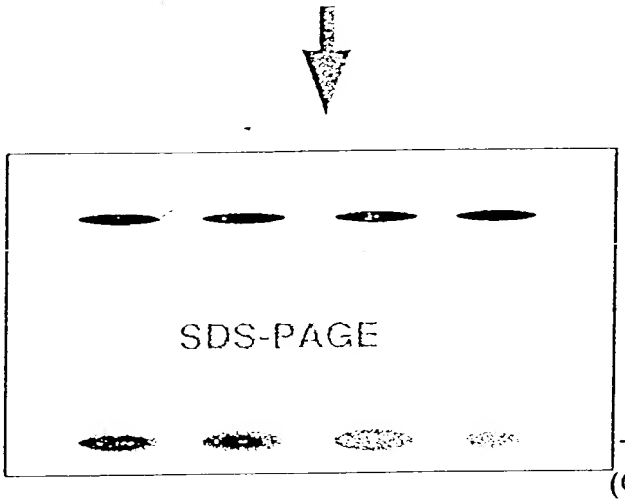
B.


-LW
-LWHM
CnA(DN) Csp1
CnB Csp1
CnA(WT)+CnB
pBridge
CnA(DN) Csp1
CnB Csp1
pBridge


A.
NF-AT
NF-AT + Csp1
–
+
iono.
B.
NF-AT
NF-AT + Csp2
+
iono.
C.
ΔCnA + NF-AT + Csp1
ΔCnA + NF-AT + Dynamin

A.

RII-peptide-PO_4 + Cn ⟶ RII-peptide + Cn + PO_4



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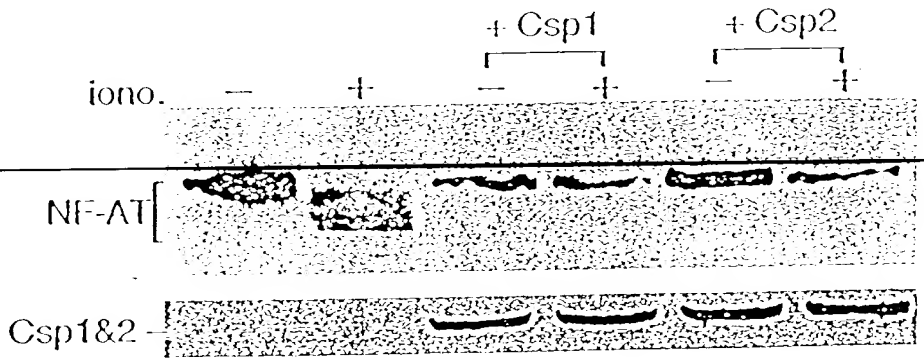

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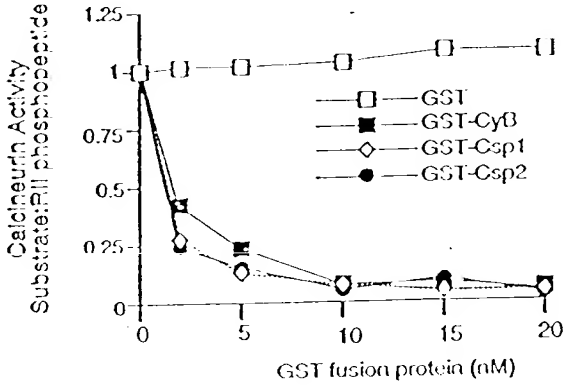
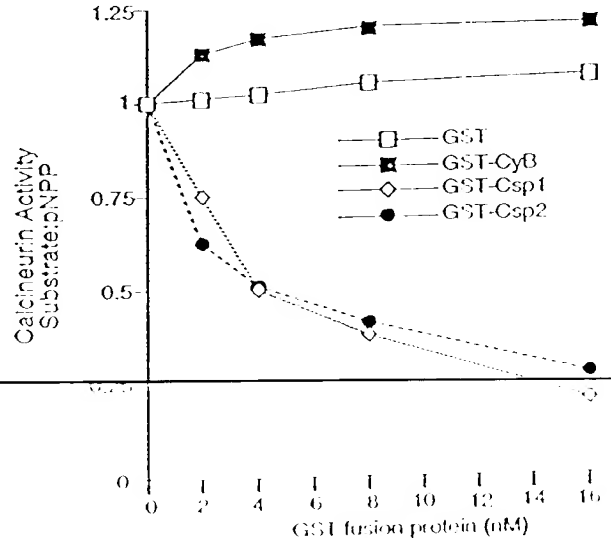
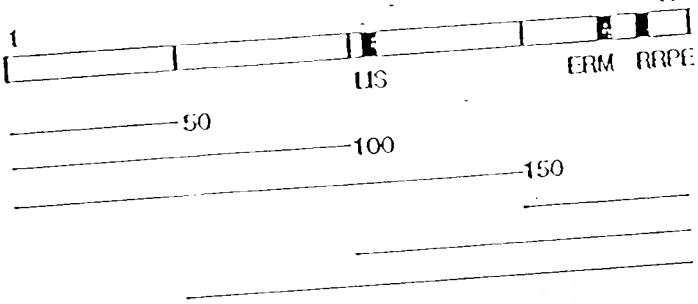

Calcineurin Activity
Substrate:RII phosphopeptide
1.25
1
0.75
0.5
0.25
0
0
5
10
15
20
GST fusion protein (nM)
GST
GST-Cyb
GST-Csp1
GST-Csp2

B.

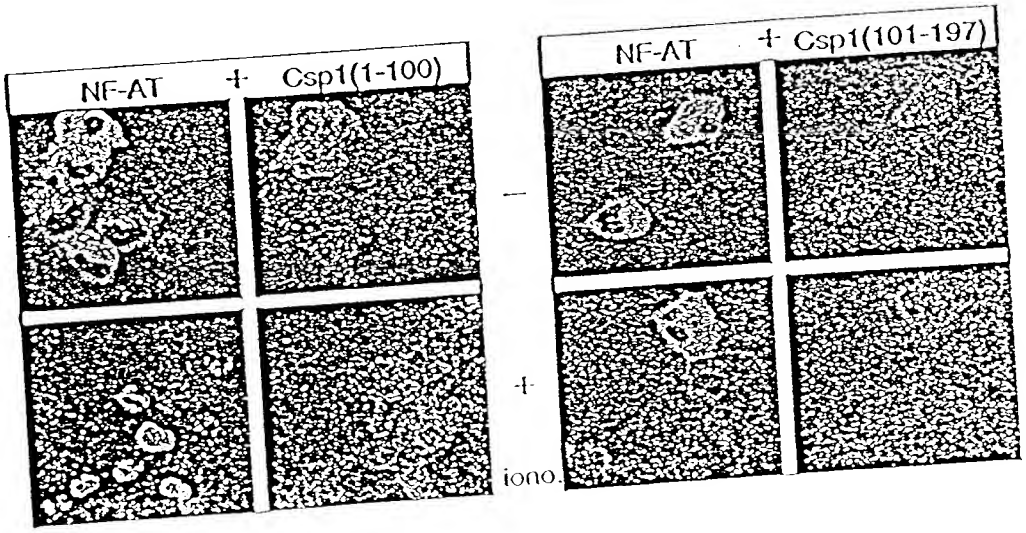

Calcineurin Activity
Substrate:pNPP
1.25
1
0.75
0.5
0
0
2
4
6
8
10
12
14
16
GST fusion protein (nM)
GST
GST-Cyb
GST-Csp1
GST-Csp2

A.



	Calcineurin binding	NF-AT inhibition
	+	+
	—	—
	—	—
	+	+/—
	+	+
	+	+
	+	+

B.



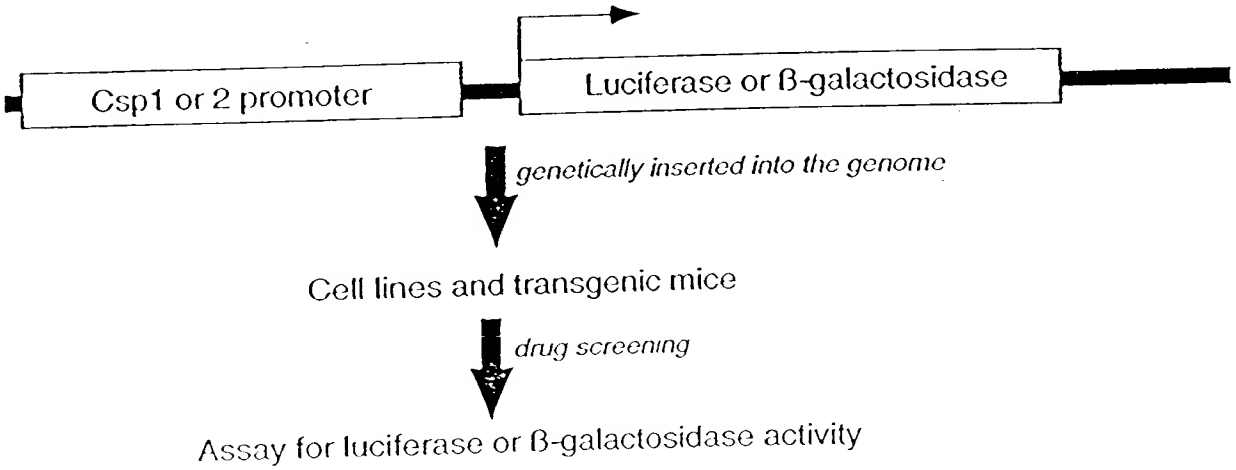
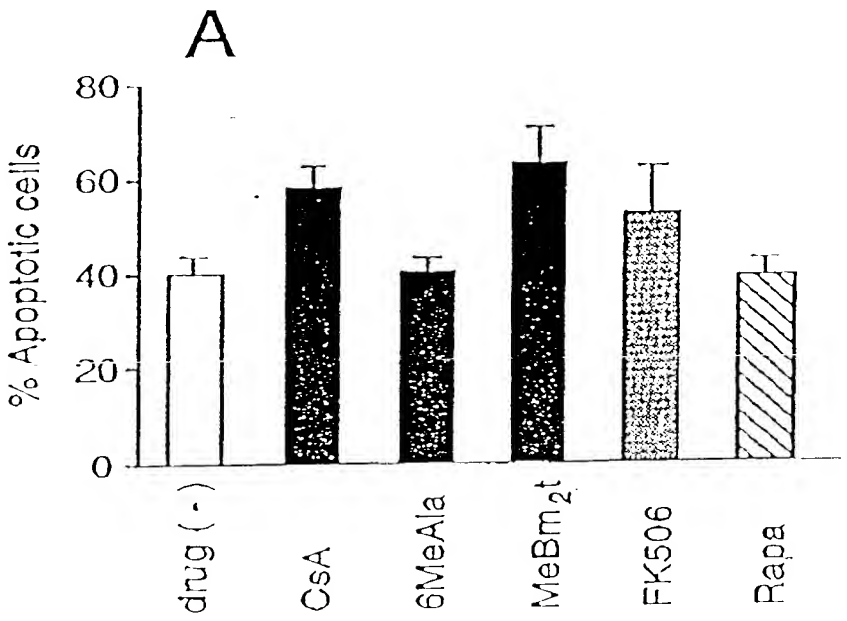
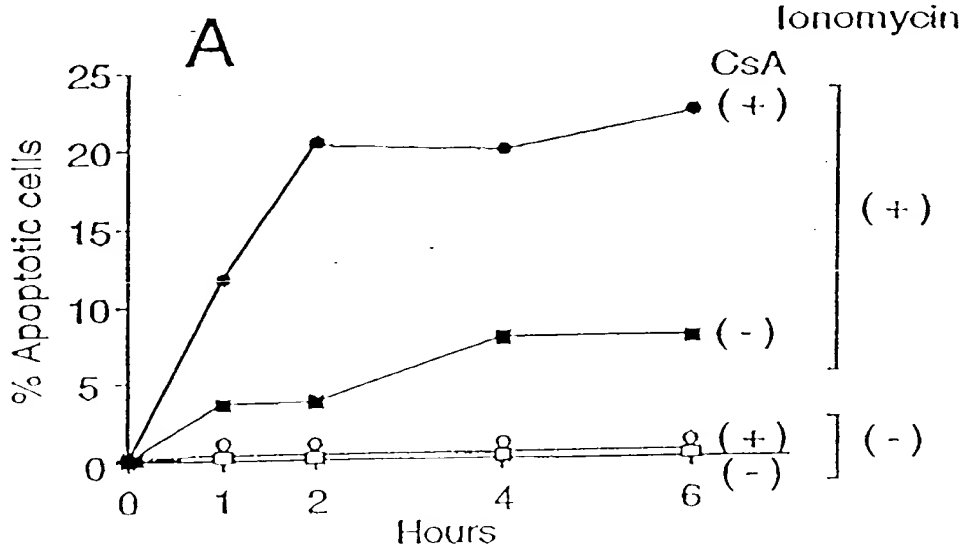
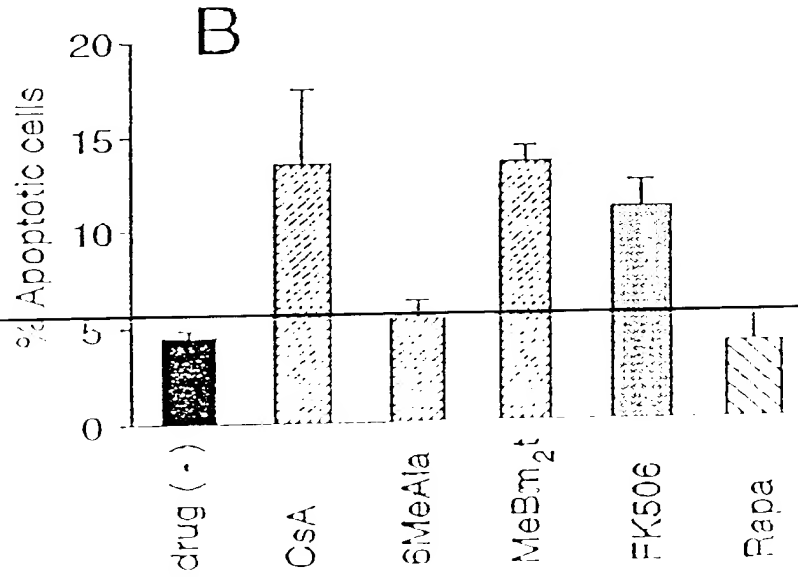

Csp1 or 2 promoter
Luciferase or ß-galactosidase
genetically inserted into the genome
Cell lines and transgenic mice
drug screening
Assay for luciferase or ß-galactosidase activity

Figure 6


A
% Apoptotic cells
80
60
40
20
0
drug (-)
CsA
6MeAla
MeBm2t
FK506
Rapa


A
Ionomycin
CsA
(+)
(-)
(+)
(-)
(+)
(-)
% Apoptotic cells
25
20
15
10
5
0
0
1
2
4
6
Hours


B
% Apoptotic cells
20
15
10
5
0
drug (-)
CsA
6MeAla
MeBm2t
FK506
Rapa

human Csp1 promoter (2.5kb) (SEQ ID NO: 1)

```
   1 cttgggttta gctccctgag gacacaaact gtcctaagac tatgataata
     gtaatcatag aaccgtgcac atggcaagtt ctgaataaat ctcagctgtt  100  MyoD
 101 ggatatactt tttgttataa ttactaacac ttcctaacta gagagtaagc
     ctactctaag aaaaaatata actgtaattt cacaacctcc aaagaaccca  200
 201 gtgcataaac agctaccatt tattaagcac tgactgaatt cttagtaata
     tgtcttcatt tttttcagat gaggaaacta agattcagct tatttgtaca  300  MyoD, NF-AT
 301 agtagttaaa aagcaaagct gaaattcaga cccaagttct cactgtatca
     tactgtccaa aaaagaattc tatttttcag gaagagacat gtctgctcac  400
 401 ttgaggtcct cttatttttc cgctattccc caaaggaaag gggtgatctc        NF-AT,NF-AT
     ttaattcttt cgttatgtcc tattgtacat agcatataat ggtaattcag  500
 501 aaaaattact tctaattaca taaattttca caatggtata gtgactaata
     cgctgaaata gaaaagtaag gcattgttat catggtctag ttcagtcttt  600  NF-AT
 601 attgcgacta tatctgataa tatacggtaa gcatctaacc acttgccagg
     ggccacagag ccacagggag actatgtctc gcttaaattc ccaaaagtgg  700
 701 gcccctgtgc ttcaaaacgt ccccgcatgg gaaccacaaa aacgttgcct
     ccccagttat caccccaagg gcccaagagc cgaggactct gcccggcgtc  800
 801 cttcagctgg caccagctgt cagaaaagcg gaactgggga cgaggacttt        MyoD
     gcccctaacc aacatggccg ccctgaggct tcgggcttcg ggcggcagaa  900
 901 ggaaggtcac gtgaagagaa ttccgttcct ttattggccc cgtctcctgg        MyoD
     aagggcgggg tacaataacc caaccggcgc cggccttaaa ggggccaccg 1000
1001 ttggatctgc cggtggccgg ccctaggggc tgggggggcg gtcgccgcgc
     cgggcttctg cccctcccgc gcggaacggt gacgggcggg gctggcgctg 1100
1101 ggaggccgtg tcgctgggag actgctgaca gcccgccgcc tgccgccgcg
     cgattccgag ggggttaacg gcggagccgc cggccgggcg cggaccggag 1200
1201 cgcgtgaggc tccggcgcgc aagcccggag cagcccgctg gggcgcacag
     ggtcgcgcgg gcgcggggat ggaggacggc gtggccggtc cccagctcgg 1300
1301 ggccgcggcg gaggcggcgg aggcggccga ggcgcgagcg cggcccgggg
     tgacgctgcg gcccttcgcg cccctctcgg gggcggccga ggcggacgag 1400
1401 ggcggcggcg actggagctt cattgactgc gagatggagg aggtggacct
     gcaggacctg cccagcgcca ccatcgcctg tcacctggac ccgcgcgtgt 1500 MyoD
1501 tcgtggacgg cctgtgccgg gtgaggaccg cgccgggcgg gccgtcgggg
     cggagggcgg acacttgttg cccgaggagg cggcgcgggt cgcagcgccc 1600 MyoD
1601 agtcccggcc gcgcgcgggg cggggaggca gcgacgtccc ccgggctgct
     cggccgcgga cccgtcaggg ctgggggcgtg gggacggcgc cccgagggtc 1700
1701 ccggtccccc agcaccccgg gggcgcgcgg agctcactgc agagtcccac
     aggctcgccc cggcccccgt gtgcgcccag gctggtgcga ctaggggggt 1800
1801 gaattcgctc cccaaggtgg ggcagcgccg ccgcccccctg cgctctcgcc
     atcgccccgc gtttactcgc tggaggaggg ggtcacctca ttcctaggga 1900
1901 ggaggaaaca gacattgagc ggcgacgtga ctcagtgttc gtaaatagga
     cgacgtccct gcattcccaa tctgcactat tggaagaaaa gccaatgttt 2000
2001 gggtgaggat ccgtggttgc tcattagcca gcggctggcc agttttggtg
     gaattgtgtt gggggggaagg ggaccatctt tcagacattt aggatatttg 2100
2101 gtcaagaacc ttgccccctt gtgtgaaggt gtggcttgcc gccatcgggg
     acacccagta catgggggagt cgactccttc ccccgcctcc ccccaccccc 2200
2201 gcaaaaatcca cacaatttag acactttgga gggtgagggg caggtatgag
     taatcaataa tggtggtggg gaggaagaat ttatttcaaa tctgcagtta 2300
2301 ttgtgcagaa taaaatgtgg acaacgtggg cgtcacagaa tgaaaccggt
     ctttgagaga tgccccatta ggaggagcagc tgtcaaaaaa agcagtgctt 2400
2401 tcagcgctta gctgtgggtc cacaaatgct gtcaatgaac tatagttgaa
     ggctgctgcc aatacaacac cactgtgaaa caga                   2484
```

Figure 9

murine Csp1 (SEQ ID NO: 2)

```
1                                                                  31
ATG GAG GAG GTG GAT CTG CAG GAC CTG CCG AGC GCC ACC ATC GCC TGC CAC CTG GAC CCG
61                                                                 91
CGC GTG TTC GTG GAC GGC CTG TGC CGG GCC AAA TTT GAA TCC CTC TTC AGA ACA TAT GAC
121                                                               151
AAG GAC ACC ACC TTC CAG TAT TTT AAG AGC TTC AAA CGT GTC CGG ATA AAC TTC AGC AAC
181                                                               211
CCC TTA TCT GCA GCC GAT GCC AGG CTG CGG CTG CAC AAG ACC GAG TTC CTG GGG AAG GAA
241                                                               271
ATG AAG TTG TAT TTT GCT CAG ACT TTA CAC ATA GGA AGT TCA CAC CTG GCT CCG CCC AAT
301                                                               331
CCC GAC AAA CAG TTC CTC ATC TCC CCT CCG GCC TCT CCT CCC GTT GGC TGG AAA CAA GTA
361                                                               391
GAA GAT GCC ACC CCC GTC ATA AAT TAC GAT CTT TTA TAT GCC ATC TCC AAG CTG GGG CCA
421                                                               451
GGA GAG AAG TAT GAA CTG CAT GCA GCG ACA GAC ACC ACT CCC AGT GTG GTG GTC CAC GTG
481                                                               511
TGT GAG AGT GAC CAA GAG AAT GAG GAG GAA GAG GAA GAG ATG GAG AGA ATG AAG AGA CCC
541                                                               571
AAG CCC AAA ATC ATC CAG ACA CGG AGA CCG GAG TAC ACA CCC ATC CAC CTC AGC TGA
```

coding sequence: 597 nucleotides

Figure 10

murine Csp2 (SEQ ID NO: 3)

```
1                                                                  31
GAA TTC GTC GAC CCA CGC GTC CGC CCA CGC GTC CGC TTG GGG CAG CAG GCA TCT ATC CCT
61                                                                 91
GAA GAT GGG GGA CTT TTC TTC CTC TGC TGC ATA GAC AGA GAC TGG GCT GTC ACT CAG TGT
121                                                               151
TTT GCT GAA GAG GCC TTC CAA GCA CTC ACT GAC TTC AGT GAT CTC CCC AAC TCA TTG TTT
181                                                               211
GCC TGC AAT GTT CAC CAG TCT GTG TTT GAA GAA GAG GAG AGC AAG GAA AAA TTC GAG GGA
241                                                               271
CTG TTC CGG ACC TAT GAT GAA TGT GTG ACG TTC CAG CTG TTT AAG AGT TTC CGA CGG GTT
301                                                               331
CGA ATA AAT TTC AGC CAT CCC AAA TCT GCA GCC CGT GCC CGG ATA GAG CTT CAT GAG ACT
361                                                               391
CAG TTC AGA GGG AAG AAG CTA AAA CTC TAC TTC GCC CAG GTC CAG ACC CCA GAG ACA GAT
421                                                               451
GGA GAC AAA CTG CAT TTG GCA CCT CCA CAG CCT GCC AAA CAG TTC CTC ATC TCA CCC CCT
481                                                               511
TCA TCT CCA TCT GTT GGC TGG AAG CCT ATC AGC GAT GCC ACA CCA GTC CTC AAC TAT GAC
541                                                               571
CTT CTT TAT GCT GTG GCC AAA CTA GGA CCA GGA GAG AAA TAT GAG CTG CAC GCT GGA ACT
601                                                               631
GAG TCT ACC CCG AGC GTC GTG GTG CAT GTG TGT GAC AGC GAC ATG GAG AGG GAG GAG GAC
661                                                               691
CCA AAG ACT TCC CCA AAG CCA AAA ATC AAT CAG ACC CGG CGG CCT GGC CTG CCA CCC TTC
721
GGT CAC TGA
```

coding sequence: 729 nucleotides

murine Csp1 (SEQ ID NO: 4)

```
1/1
ATG GAG GAG GTG GAT CTG CAG GAC CTG CCG
M   E   E   V   D   L   Q   D   L   P
31/11
AGC GCC ACC ATC GCC TGC CAC CTG GAC CCG
S   A   T   I   A   C   H   L   D   P
61/21
CGC GTG TTC GTG GAC GGC CTG TGC CGG GCC
R   V   F   V   D   G   L   C   R   A
91/31
AAA TTT GAA TCC CTC TTC AGA ACA TAT GAC
K   F   E   S   L   F   R   T   Y   D
121/41
AAG GAC ACC ACC TTC CAG TAT TTT AAG AGC
K   D   T   T   F   Q   Y   F   K   S
151/51
TTC AAA CGT GTC CGG ATA AAC TTC AGC AAC
F   K   R   V   R   I   N   F   S   N
181/61
CCC TTA TCT GCA GCC GAT GCC AGG CTG CGG
P   L   S   A   A   D   A   R   L   R
211/71
CTG CAC AAG ACC GAG TTC CTG GGG AAG GAA
L   H   K   T   E   F   L   G   K   E
241/81
ATG AAG TTG TAT TTT GCT CAG ACT TTA CAC
M   K   L   Y   F   A   Q   T   L   H
271/91
ATA GGA AGT TCA CAC CTG GCT CCG CCC AAT
I   G   S   S   H   L   A   P   P   N
301/101
CCC GAC AAA CAG TTC CTC ATC TCC CCT CCG
P   D   K   Q   F   L   I   S   P   P
331/111
GCC TCT CCT CCC GTT GGC TGG AAA CAA GTA
A   S   P   P   V   G   W   K   Q   V
361/121
GAA GAT GCC ACC CCC GTC ATA AAT TAC GAT
E   D   A   T   P   V   I   N   Y   D
391/131
CTT TTA TAT GCC ATC TCC AAG CTG GGG CCA
L   L   Y   A   I   S   K   L   G   P
421/141
GGA GAG AAG TAT GAA CTG CAT GCA GCG ACA
G   E   K   Y   E   L   H   A   A   T
451/151
GAC ACC ACT CCC AGT GTG GTG GTC CAC GTG
D   T   T   P   S   V   V   V   H   V
481/161
TGT GAG AGT GAC CAA GAG AAT GAG GAG GAA
C   E   S   D   Q   E   N   E   E   E
511/171
GAG GAA GAG ATG GAG AGA ATG AAG AGA CCC
E   E   E   M   E   R   M   K   R   P
541/181
AAG CCC AAA ATC ATC CAG ACA CGG AGA CCG
K   P   K   I   I   Q   T   R   R   P
571/191
GAG TAC ACA CCC ATC CAC CTC AGC TGA
E   Y   T   P   I   H   L   S   *
```

198 amino acids and 597 nucleotides

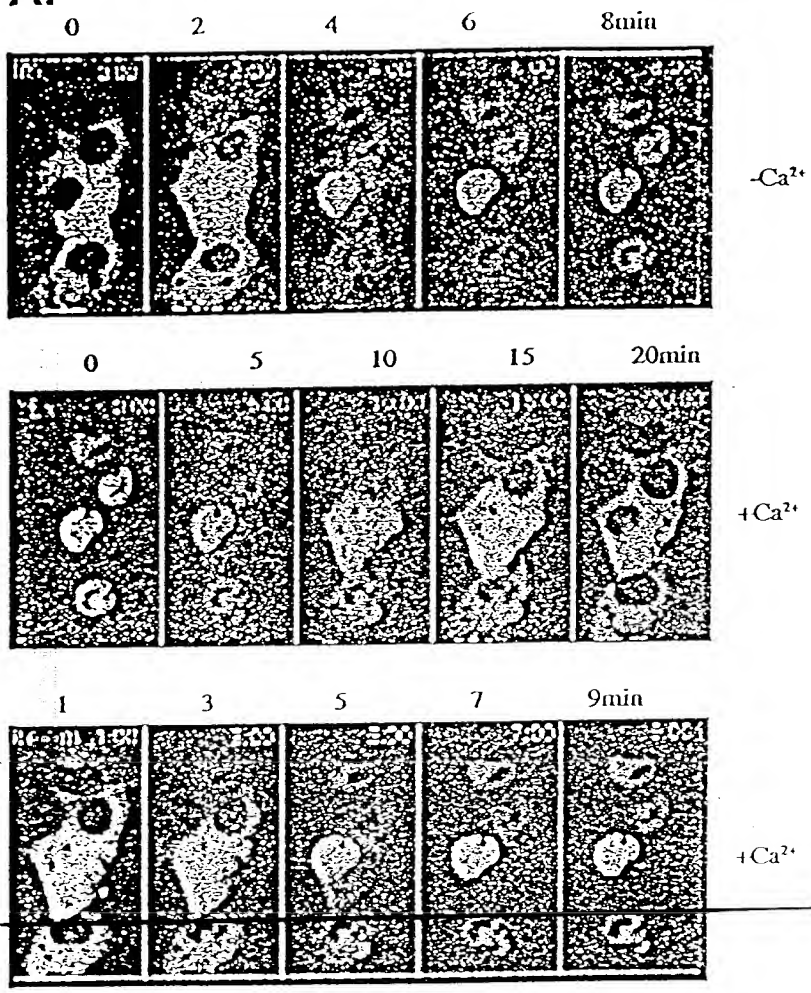
murine Csp2 (S. . ID NO: 5)

```
1/1                                                                 31/11
GAA TTC GTC GAC CCA CGC GTC CGC CCA CGC GTC CGC TTG GGG CAG CAG GCA TCT ATC CCT
E   F   V   D   P   R   V   R   P   R   V   F   L   G   Q   Q   A   S   I   P
61/21                                                               91/31
GAA GAT GGG GGA CTT TTC TTC CTC TGC TGC ATA GAC AGA GAC TGG GCT GTC ACT CAG TGT
E   D   G   G   L   F   F   L   C   C   I   D   R   D   W   A   V   T   Q   C
121/41                                                              151/51
TTT GCT GAA GAG GCC TTC CAA GCA CTC ACT GAC TTC AGT GAT CTC CCC AAC TCA TTG TTT
F   A   E   E   A   F   Q   A   L   T   D   F   S   D   L   P   N   S   L   F
181/61                                                              211/71
GCC TGC AAT GTT CAC CAG TCT GTG TTT GAA GAA GAG GAG AGC AAG GAA AAA TTC GAG GGA
A   C   N   V   H   Q   S   V   F   E   E   E   E   S   K   E   K   F   E   G
241/81                                                              271/91
CTG TTC CGG ACC TAT GAT GAA TGT GTG ACG TTC CAG CTG TTT AAG AGT TTC CGA CGG GTT
L   F   R   T   Y   D   E   C   V   T   F   Q   L   F   K   S   F   R   R   V
301/101                                                             331/111
CGA ATA AAT TTC AGC CAT CCC AAA TCT GCA GCC CGT GCC CGG ATA GAG CTT CAT GAG ACT
R   I   N   F   S   H   P   K   S   A   A   R   A   R   I   E   L   H   E   T
361/121                                                             391/131
CAG TTC AGA GGG AAG AAG CTA AAA CTC TAC TTC GCC CAG GTC CAG ACC CCA GAG ACA GAT
Q   F   R   G   K   K   L   K   L   Y   F   A   Q   V   Q   T   P   E   T   D
421/141                                                             451/151
GGA GAC AAA CTG CAT TTG GCA CCT CCA CAG CCT GCC AAA CAG TTC CTC ATC TCA CCC CCT
G   D   K   L   H   L   A   P   P   Q   P   A   K   Q   F   L   I   S   P   P
481/161                                                             511/171
TCA TCT CCA TCT GTT GGC TGG AAG CCT ATC AGC GAT GCC ACA CCA GTC CTC AAC TAT GAC
S   S   P   S   V   G   W   K   P   I   S   D   A   T   P   V   L   N   Y   D
541/181                                                             571/191
CTT CTT TAT GCT GTG GCC AAA CTA GGA CCA GGA GAG AAA TAT GAG CTG CAC GCT GGA ACT
L   L   Y   A   V   A   K   L   G   P   G   E   K   Y   E   L   H   A   G   T
601/201                                                             631/211
GAG TCT ACC CCG AGC GTC GTG GTG CAT GTG TGT GAC AGC GAC ATG GAG AGG GAG GAG GAC
E   S   T   P   S   V   V   V   H   V   C   D   S   D   M   E   R   E   E   D
661/221                                                             691/231
CCA AAG ACT TCC CCA AAG CCA AAA ATC AAT CAG ACC CGG CGG CCT GGC CTG CCA CCC TTC
P   K   T   S   P   K   P   K   I   N   Q   T   R   R   P   G   L   P   P   F
721/241
GGT CAC TGA
G   H   *
```

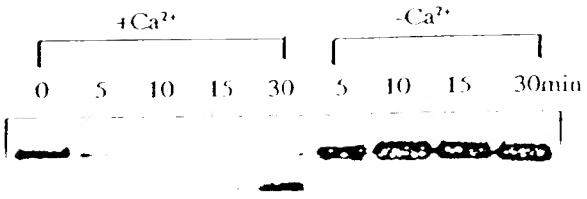
242 amino acids and 729 nucleotides

FIGURE 1

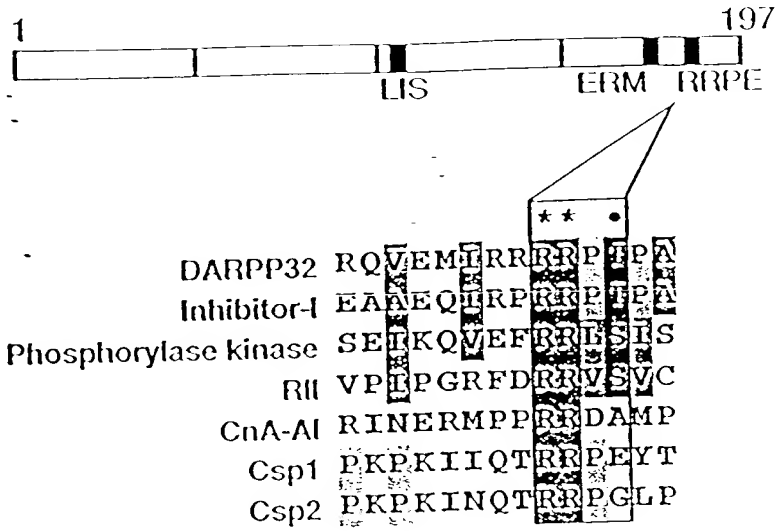
A.


0
2
4
6
8min
-Ca2+
0
5
10
15
20min
+Ca2+
1
3
5
7
9min
+Ca2+

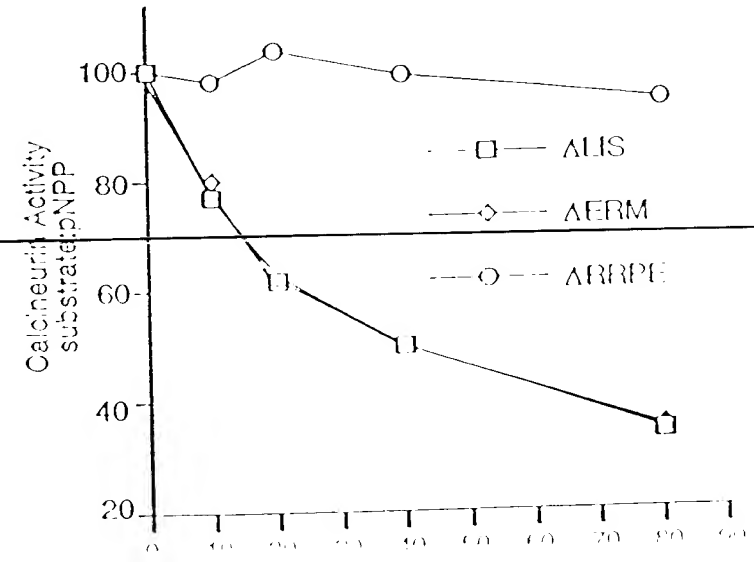
B.

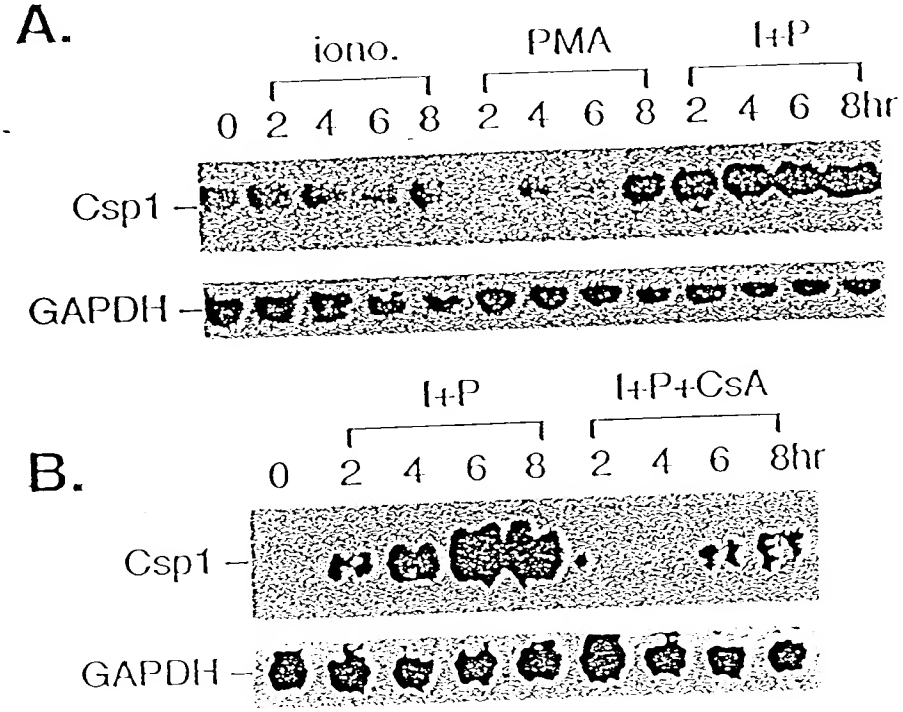

+Ca2+
-Ca2+
0
5
10
15
30
5
10
15
30min

A.


1
197
LIS
ERM
RRPE
DARPP32 RQVEMTRRRRPTPA
Inhibitor-I EAAEQTRPRRPTPA
Phosphorylase kinase SETKQVEFRRLSIS
RII VPTPGRFDRRVSVC
CnA-AI RINERMPPRRDAMP
Csp1 PKPKIIQTRRPEYT
Csp2 PKPKINQTRRPGLP

B.


100
80
60
40
20
Calcineurin Activity
substrate:pNPP
ALIS
AERM
ARRPE


A.
iono.
PMA
I+P
0 2 4 6 8 2 4 6 8 2 4 6 8hr
Csp1
GAPDH
B.
I+P
I+P+CsA
0 2 4 6 8 2 4 6 8hr
Csp1
GAPDH

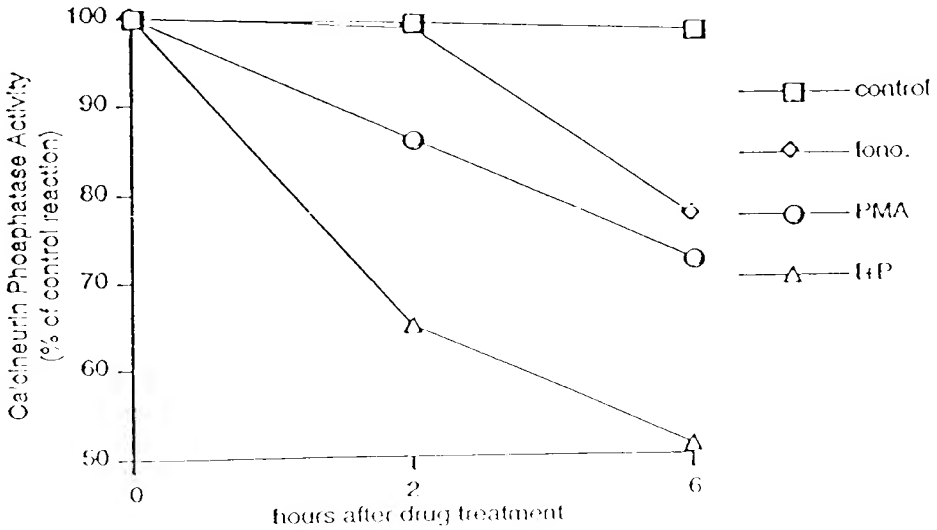

Calcineurin Phoephatase Activity
(% of control reaction)
100
90
80
70
60
50
0
2
6
hours after drug treatment
control
iono.
PMA
I+P

Figure 18

Murine Csp3 (SEQ ID No: 22)
cDNA Nucleic acid sequence (coding)

atgctccgagacagcctgaaatcttggaatgacagccagtcagacctctgtagcagcgaccaggaggaggaagaggagatggtcttcggt
gaaaatgaggacggactggaagagatgatggacctaagtgacctgcccacctcactctttgcttgcagtgtccatgaagcagtgtttgaggt
ccaagagcaaaaggagaggtttgaggccctgttcaccctctacgatgaccaggtcacattccagttgttcaagagttttcgcagagtgaggat
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tgcacgcgggaaccgagtccaccccccagtgtggtggtgcacgtctgtgagagcgaaactgaagaggaagaagacacaaaaaatccaaaa
cagaaaatcacgcagacgcggcgcccggaggctcccacggcggcactgagtgagcggctggactgtgcactctga

Figure 19

cDNA nucleic acid sequence
(entire coding + 5' and 3' UTR) (SEQ ID No: 23)

gccgctgcggcccgcgttgagggcgtggtggctccgggtggtgagggtctgtccgccccaggccgcgctcgtggg
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tccatgaagcagtgtttgaggtccaagagcaaaaggagaggtttgaggccctgttcaccctctacgatgaccaggtca
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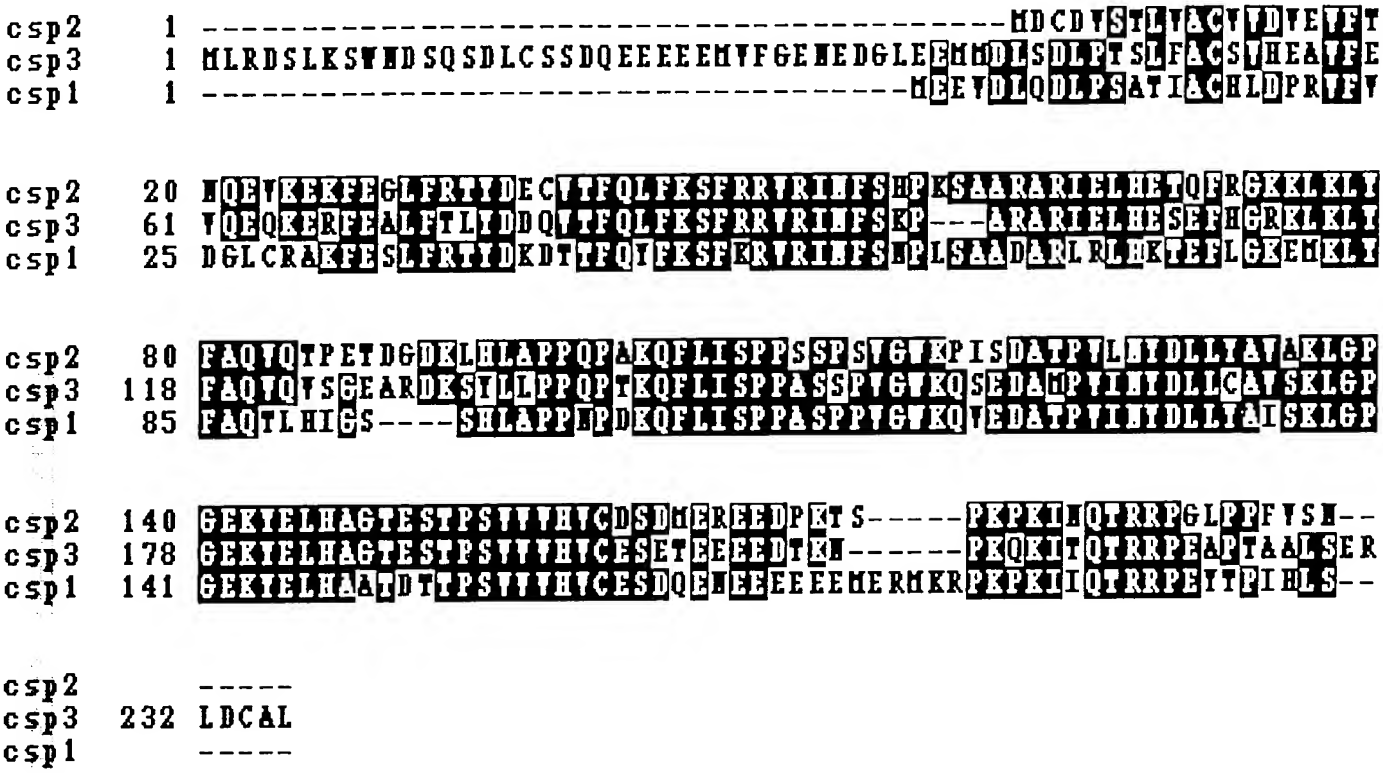
gaagatgcgatgccagtgatcaactatgacctgctctgcgctgtctccaagctgggcccaggggagaaatacgaact
gcacgcgggaaccgagtccacccccagtgtggtggtgcacgtctgtgagagcgaaactgaagaggaagaagacac
aaaaaatccaaaacagaaaatcacgcagacgcggcgcccggaggctcccacggcggcactgagtgagcggctgg
actgtgcactctgagcggctgcggtgcctgccgcgcctgcctgtcccaccactacagctgcgcctgtctaggagcaca
gcccagggatgctcttgcatccgtcag

Figure 20

Murine Csp3 (SEQ ID NO: 24)
Amino acid sequence

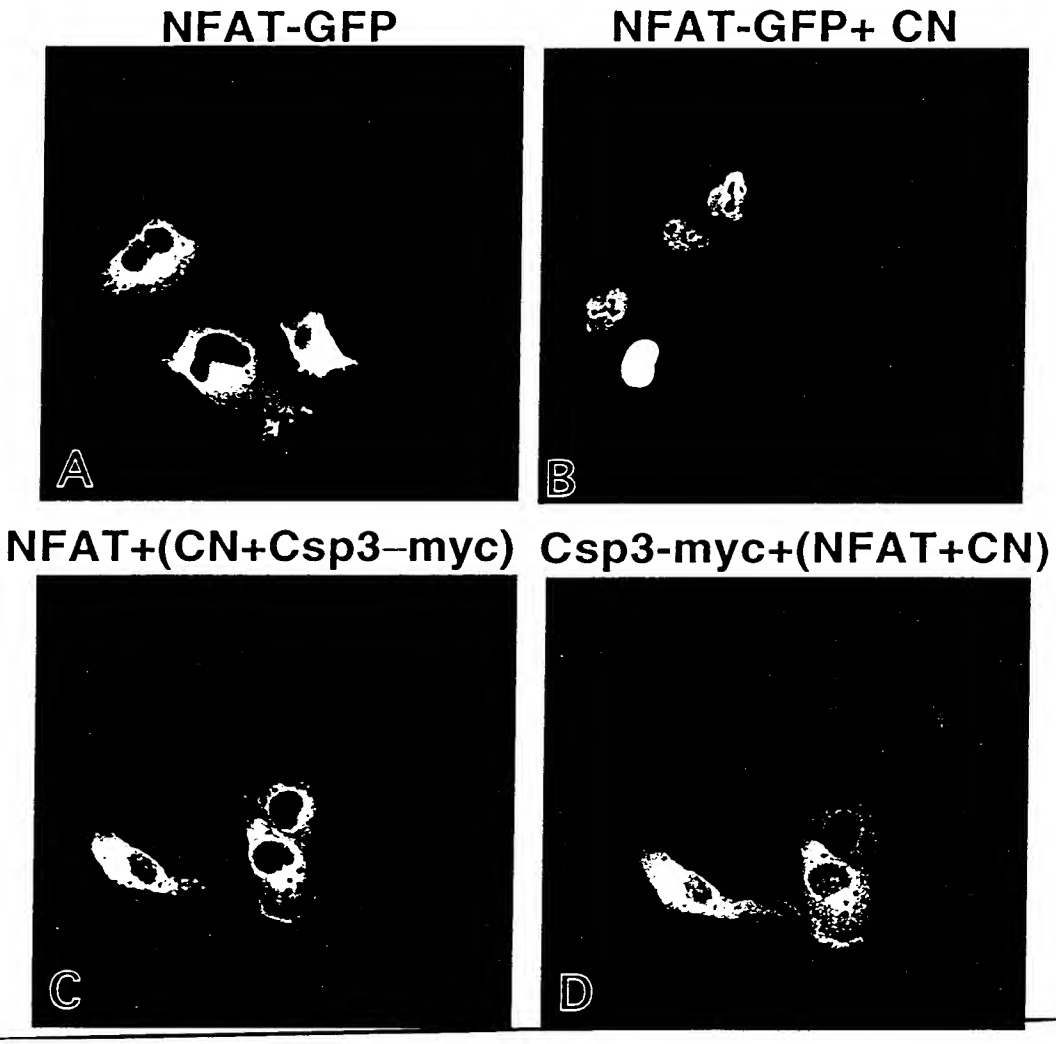
MLRDSLKSWNDSQSDLCSSDQEEEEEMVFGENEDGLEEMMDLSDLPTSLFACSVHEAV
FEVQEQKERFEALFTLYDDQVTFQLFKSFRRVRINFSKPARARIELHESEFHGRKLKLYF
AQVQVSGEARDKSYLLPPQPTKQFLISPPASSPVGWKQSEDAMPVINYDLLCAVSKLGP
GEKYELHAGTESTPSVVVHVCESETEEEEDTKNPKQKITQTRRPEAPTAALSERLDCALZ

Figure 21 **Identification of a Third Calcipressin Family Member, Csp3**



A third calcipressin family member, termed csp3, was cloned from murine T cells and found to have high sequence homology with csp1 and csp2.

Figure 22 **Calcipressin 3 Inhibits Calcineurin Mediated Translocation of NFAT**

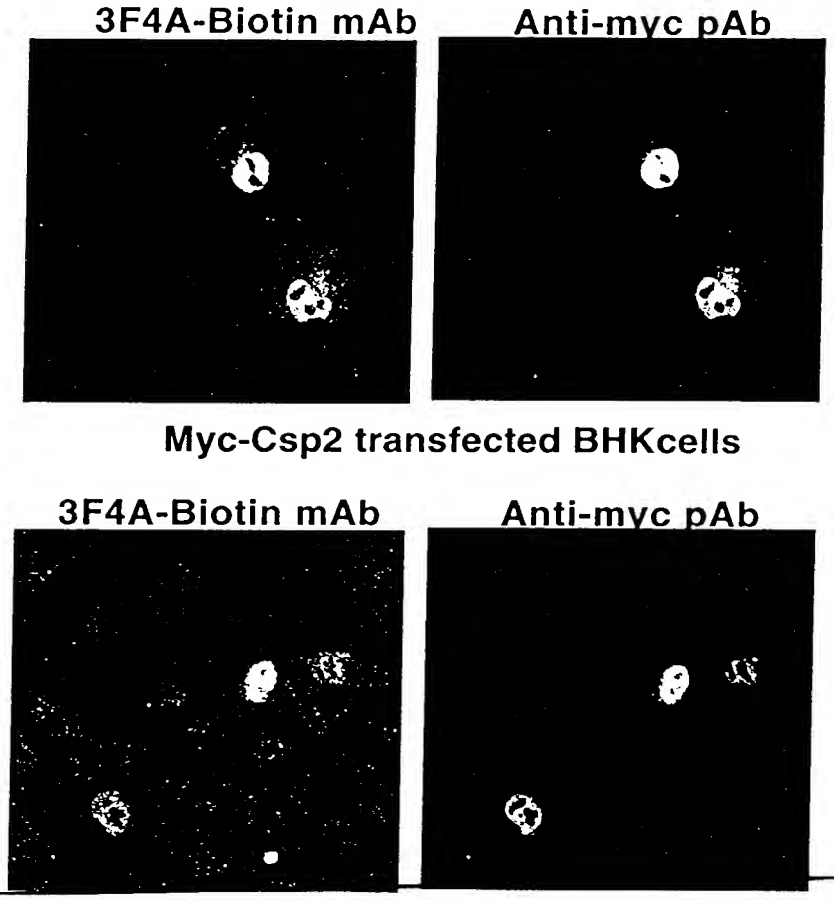


Panel A demonstrates the cytoplasmic expression pattern of the transcription factor NFAT tagged with green fluorescent protein (GFP) in the absence of stimulus. Upon co-expression of calcineurin (CN), NFAT shuttles into the nucleus as seen in panel B.

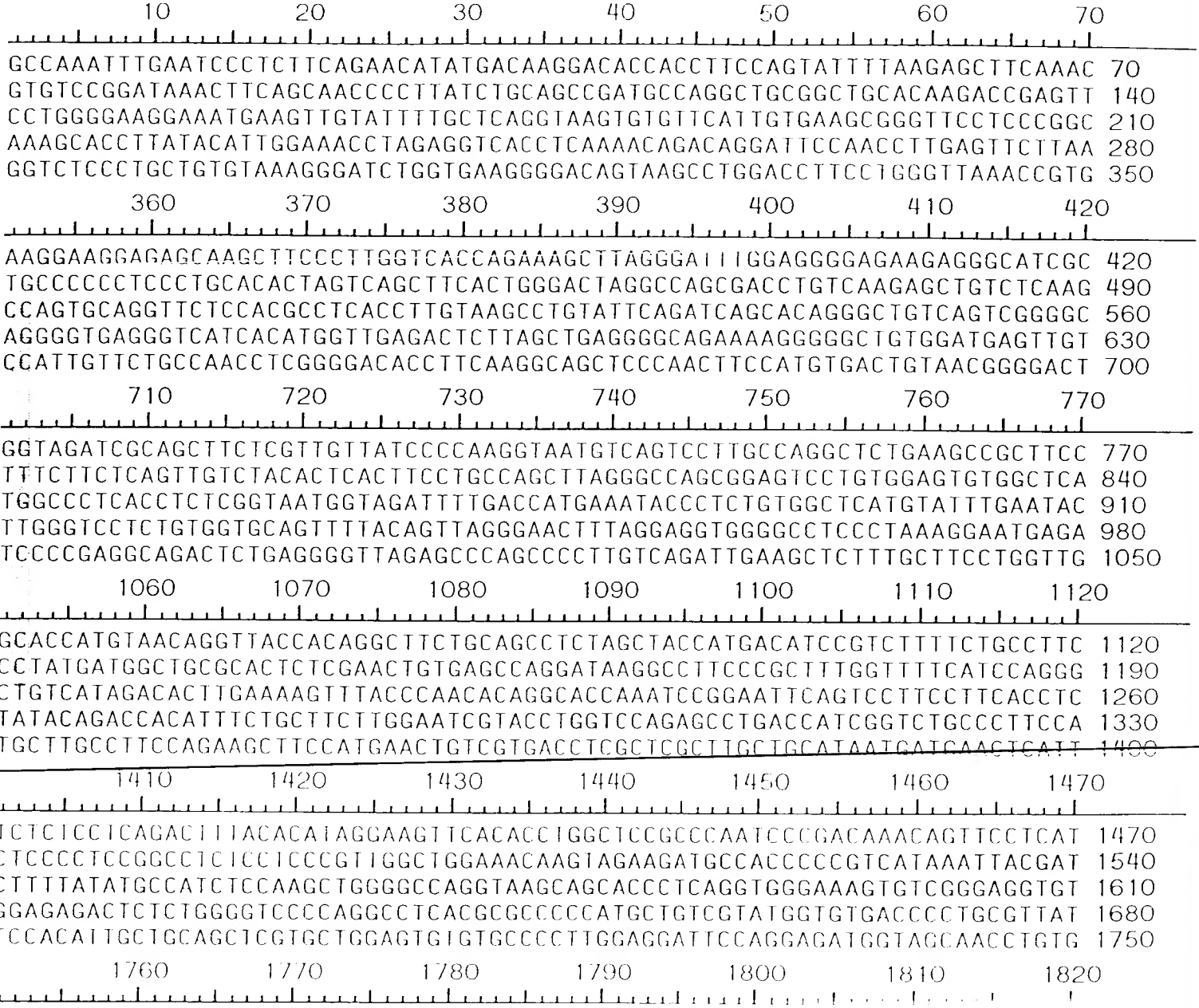
Panel C demonstrates the cytoplasmic expression of NFAT in the presence of calcineurin and calcipressin 3 (Csp3), suggesting inhibition of CN activity

[illegible] antibody to detect the myc-tagged Csp3 protein.

Figure 23 . **Generation of anti-Csp2 and anti-Csp1 Monoclonal Antibodies**



Monoclonal antibodies (mAb) were generated against Csp1 and Csp2. 3F4A mAb was biotinylated and demonstrated to recognize cells transfected with both myc-tagged csp2 (top panel) and csp1 (bottom panel), as verified by immunostaining with a myc pAb.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CCTGGGGAAGGAAATGAAGTTGTATTTTGCTCAGGTAAGTGTGTTCATTGTGAAGCGGGTTCCTCCCGGC 210
AAAGCACCTTATACATTGGAAACCTAGAGGTCACCTCAAAACAGACAGGATTCCAACCTTGAGTTCTTAA 280
GGTCTCCCTGCTGTGTAAAGGGATCTGGTGAAGGGGACAGTAAGCCTGGACCTTCCTGGGTTAAACCGTG 350
360 370 380 390 400 410 420
AAGGAAGGAGAGCAAGCTTCCCTTGGTCACCAGAAAGCTTAGGGATTTGGAGGGGAGAAGAGGGCATCGC 420
TGCCCCCCTCCCTGCACACTAGTCAGCTTCACTGGGACTAGGCCAGCGACCTGTCAAGAGCTGTCTCAAG 490
CCAGTGCAGGTTCTCCACGCCTCACCTTGTAAGCCTGTATTCAGATCAGCACAGGGCTGTCAGTCGGGGC 560
AGGGGTGAGGGTCATCACATGGTTGAGACTCTTAGCTGAGGGGCAGAAAAGGGGGCTGTGGATGAGTTGT 630
CCATTGTTCTGCCAACCTCGGGGACACCTTCAAGGCAGCTCCCAACTTCCATGTGACTGTAACGGGGACT 700
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TGGCCCTCACCTCTCGGTAATGGTAGATTTTGACCATGAAATACCCTCTGTGGCTCATGTATTTGAATAC 910
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1060 1070 1080 1090 1100 1110 1120
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1410 1420 1430 1440 1450 1460 1470
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1760 1770 1780 1790 1800 1810 1820

Figure 24

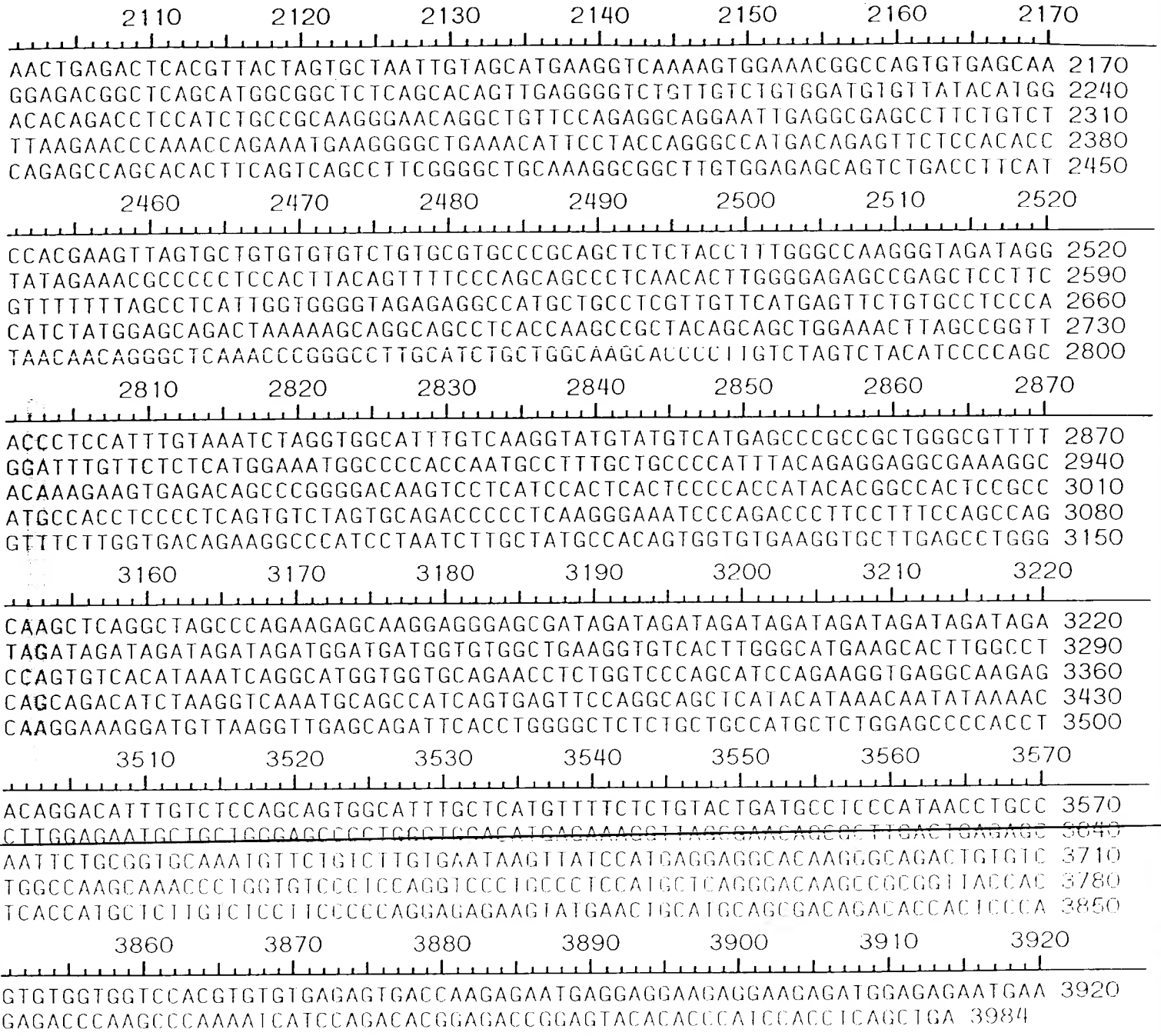
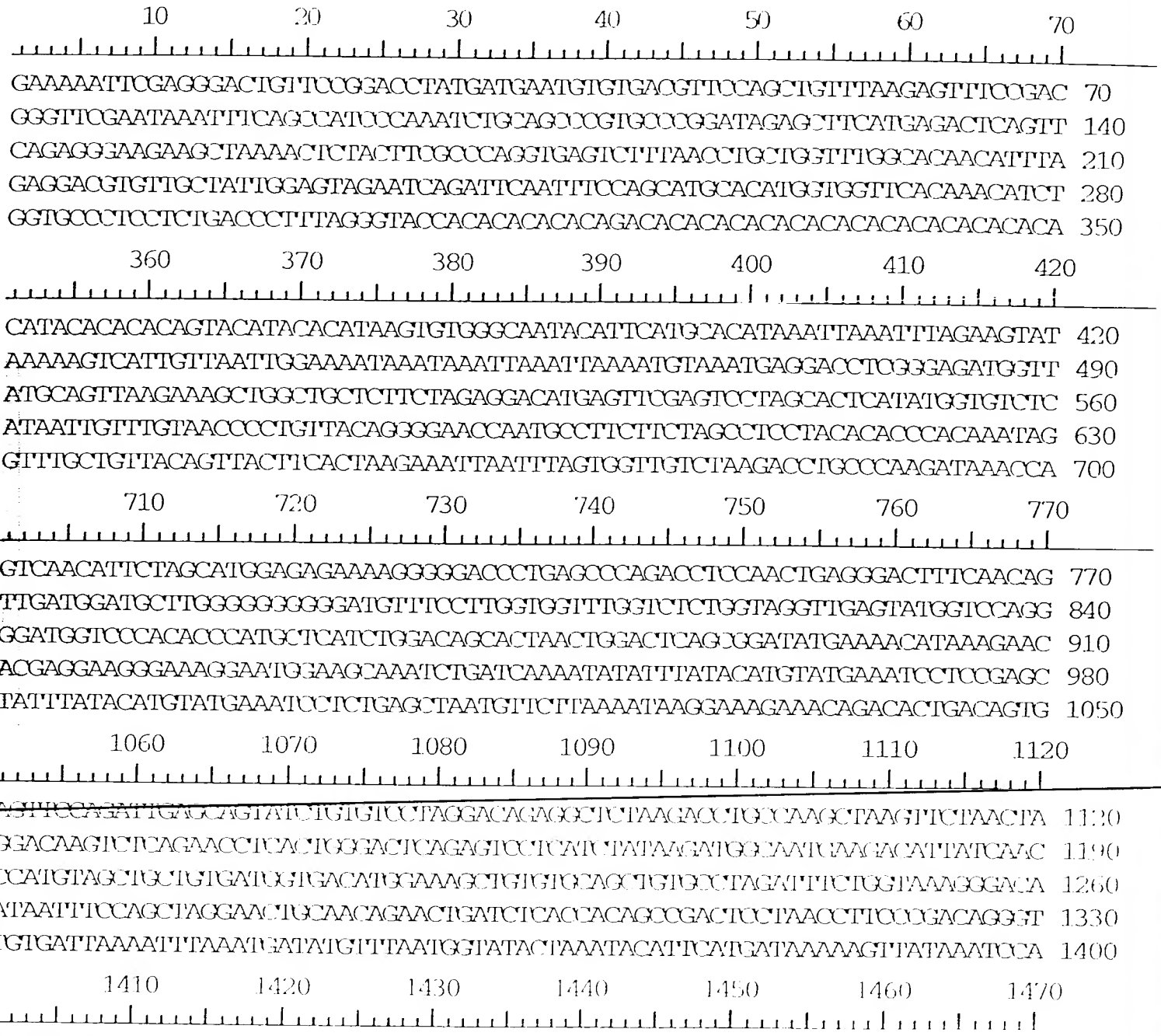


Figure 3A Continued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CAGAGGGAAGAAGCTAAAACTCTACTTCGCCCAGGTGAGTCTTTAACCTGCTGGTTTGGCACAACATTTA 210
GAGGACGTGTTGCTATTGGAGTAGAATCAGATTCAATTTCCAGCATGCACATGGTGGTTCACAAACATCT 280
GGTGCCCTCCTCTGACCCTTTAGGGTACCACACACACACAGACACACACACACACACACACACACACA 350
360 370 380 390 400 410 420
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ATAATTGTTTGTAACCCCTGTTACAGGGGAACCAATGCCTTCTTCTAGCCTCCTACACACCCACAAATAG 630
GTTTGCTGTTACAGTTACTTCACTAAGAAATTAATTTAGTGGTTGTCTAAGACCTGCCCAAGATAAACCA 700
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1060 1070 1080 1090 1100 1110 1120
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1410 1420 1430 1440 1450 1460 1470

Figure 25

1760 1770 1780 1790 1800 1810 1820

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ATGATACATACATACATGATAGACACATACATAGATACATACATAGCTATATAGATACGAGAGAGAGACA 2030
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2110 2120 2130 2140 2150 2160 2170

AAAATCATGAACTCAAACAAATAGTTAAGCAAGAATTCACAGCAGCACTGTTCACAATAGGCCAACAGTG 2170
AGAACTACCTAAAGATCTTCAACAGATAAAGGGATAAAGAGACAATAGTATGTTCACACAAAGGAATATT 2240
ATTCAGCTGAGAGAGAGAGAGAGAGAGATGTTGATAATCCATCACCAAATAATGGGCCTTTAAAATGCAA 2310
TGGAAGCTAGACACAAAAGCTCATCTGTTCTGTGGTTCCATTCTCATAAAAGAGTTAGATAAGTTCAGAG 2380
AAGTAGACACAGCTTGACAACCATCAGGGGTAGTAGGAAACTACATTAGTAGTCGTTATTTAAGGGATGC 2450

2460 2470 2480 2490 2500 2510 2520

ATAGTTGGGGTTGGAGAGATGACTTAGCAGTTAAGAGCACTGAATGCTCTTTCGAAGGCCCTGAGTTCAA 2520
ATCCCAGCAACCATATGGTGGCTCACAACCATCCATAATGAGATCTGATGCCCTTTTCTGGAGTGTCTGG 2590
AGACAGCTACCATGTACTCACATATAATAAATAAATAAATCTTTTTAAAAAAGGGGTGTGTGTGTGCATA 2660
GTTATCCTTAGAGCCATGGGAAGGGTTAGGGTAGTTGTTCTCAACCTTCCTAATGCTGTGACCCTTTAAT 2730
ACTCATGAAGTGGTGATCCCCAGCCACAAAATCCTTTTCGTTGCTGCTTTATAACTGTAATTTTGCTAGT 2800

2810 2820 2830 2840 2850 2860 2870

ACTATGAATTATGATACCACTGTGTGTGTTTTCTGATGGTCTTAGGCAGCACTCTGGCTTGCTCACCATC 2870
TAGCCTAACCTTATTGGTGACATCAAGGTTTTTTGGGTTGTTTTTTTTTTAAAGGGCACTGTTACCTAAGA 2940
AGGACATTAGAAGTTGTCCACTAGCTTCCACATGTACACACTCATAAGGCACACAATGTAGTACAGGGC 3010
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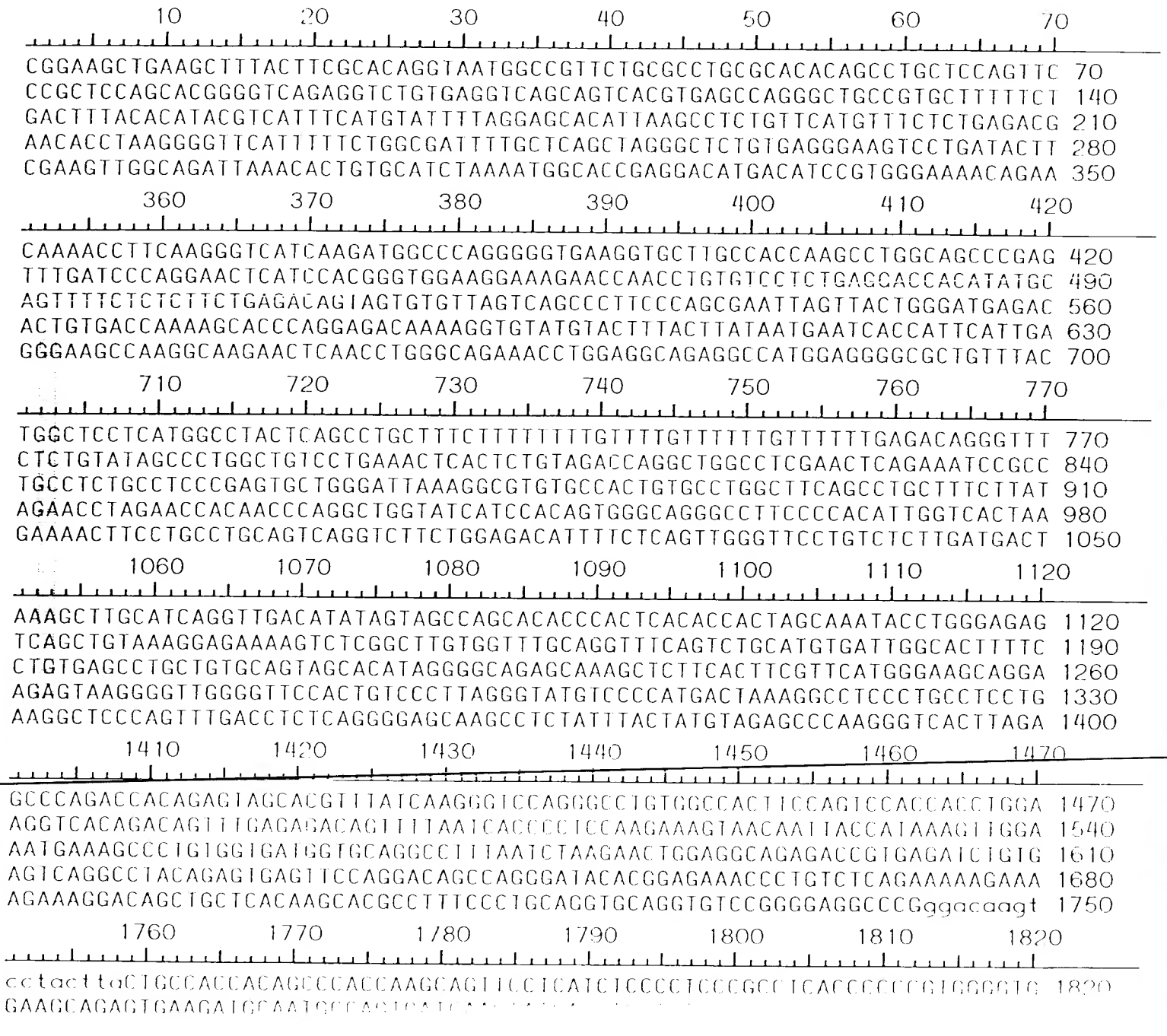
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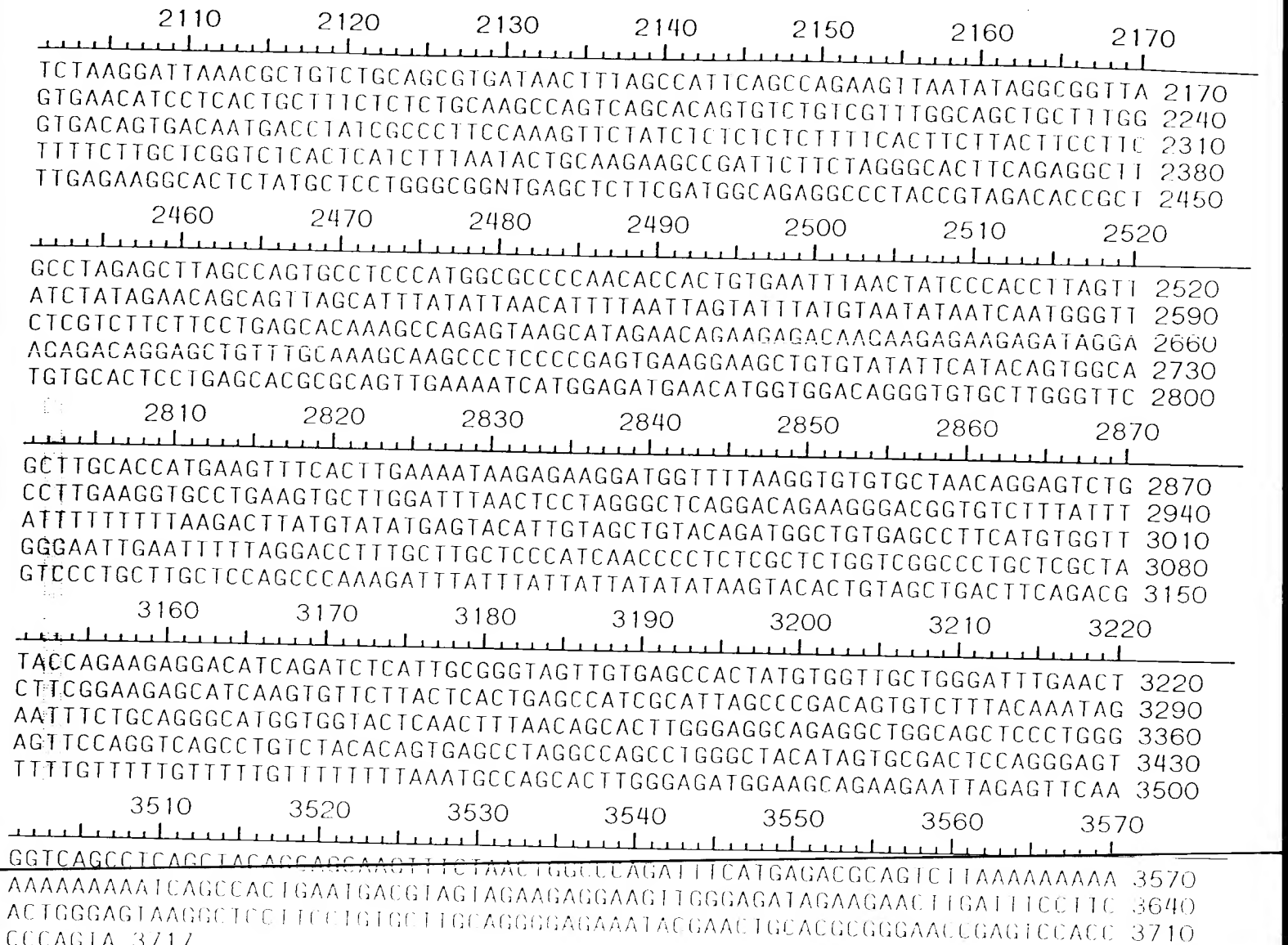
Figure 2b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2810 2820 2830 2840 2850 2860 2870
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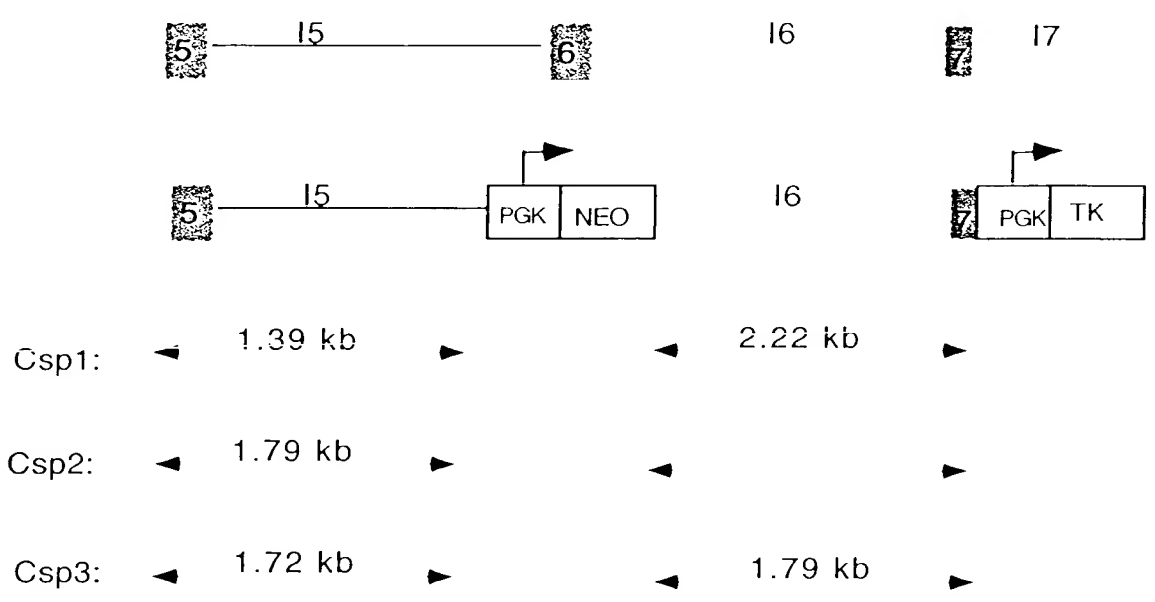
CCCAGTA 3717

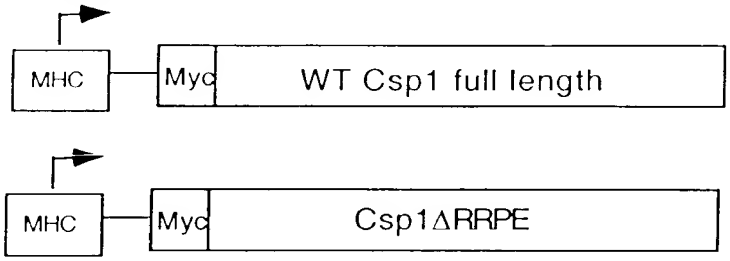
Figure 27 . **Schematic Representation of the Gene-targeting Vectors Used to Disrupt the Csp1, -2, and -3 Genes**



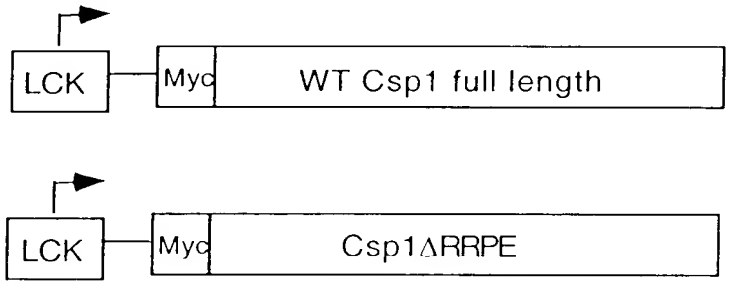
This schematic diagram shows the organization of the Csp genes (top) and the targeting vectors (middle)constructed to disrupt the Csp genes. Our targeting vector will replace exon 6 with the neomycin drug resistance genes. This exon contains the start of the inhibitory, or c-terminal domain of all three genes which should effectively destroy the calcineurin inhibition activity. The genomic structure of all three genes is relatively similar with different size introns (I5, I6). Exons are denoted by the shaded boxes with numbers.

Figure 28 **Constructs Used to Generate Tissue-Specific Expression of Csp1 in Transgenic Mice**

Cardiac Specifc Expression:



T-Cell Specific Expression:



This schematic diagram demonstrates the constructs injected into blastocysts to generate transgenic mice. Wild-type full length myc-tagged Csp1 under the control of a myosin heavy chain (MHC) promoter (top half) will ensure cardiac specific expression. Similarly Csp1 with the sequence element, amino acids,188-191,"RRPE" deleted is also expressed under the MHC promoter.